Research

Multidrug-Resistant Tuberculosis in Prison Inmates, Azerbaijan

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In a tuberculosis (TB) program in the Central Penitentiary Hospital of Azerbaijan, we analyzed 65 isolates of *Mycobacterium tuberculosis* by IS*6110*-based restriction fragment-length polymorphism (RFLP) and spoligotyping. From 11 clusters associated with 33 patients, 31 isolates had an IS*6110*-based banding pattern characteristic of the Beijing genotype of *M. tuberculosis*. In addition, 15 *M. tuberculosis* isolates with similar RFLP patterns constituted a single group by spoligotyping, matching the Beijing genotype. Multidrug resistance, always involving isoniazid and rifampin, was seen in 34 (52.3%) of 65 isolates, with 28 belonging to the Beijing genotype.

Tuberculosis (TB) is an important health problem in prison settings (1), where factors such as poor general health of inmates, overcrowding, increased risk factors, delayed case finding, and incomplete or inadequate therapeutic intervention contribute to rapid spread (2) and high prevalence (e.g., 500 cases per 100,000 inmates in New York City jails [3] and 2,283 per 100,000 inmates in the General Penitentiary Hospital in Madrid, Spain [4]). In prisons of the former Soviet Union, TB incidence is even higher (e.g., in Siberia [5] or Russia as a whole [6,7]). TB incidence is also associated with length of incarceration (3). Transmission patterns are generally difficult to establish (8), and the rate of unrecognized transmission may be quite high (9). Above all, strains isolated in these settings are often multidrug resistant (MDR) (10,11).

According to the International Committee of the Red Cross (ICRC), the total number of inmates in the Azerbaijan prison system is approximately 25,000. With 4,667 TB cases per 100,000, the incidence in Azeri prisons is nearly 50 times higher than the country average, and the mortality rate may reach 24% (6). In June 1995, the ICRC implemented a TB control program in the Central Penitentiary Hospital in Baku, Azerbaijan, which was the only treatment center for Azeri prisoners with TB. The program was based on directly observed therapy, short course (DOTS), as recommended by the World Health Organization (WHO [12]) and the International Union Against Tuberculosis and Lung Disease (IUATLD [13]). After treatment failures were observed despite completion of strictly supervised therapy, susceptibility testing of inmates' *M. tuberculosis* isolates demonstrated a high rate of multidrug resistance (11).

Several outbreaks of MDR-TB have been reported worldwide from hospitals (affecting patients and health-care workers [14]), the community (15), and prisons (4). In these outbreaks, MDR-TB has predominantly affected immunocompromised patients, and disease was often caused by certain strains of *M. tuberculosis*, in particular the W strain (16-18). This strain and its many variants (e.g., subtypes W_1 [17] and U [19]), form a family lineage that shares a common genotype with the Beijing clone (20). At the molecular level, they exhibit a closely related, unique multiband IS*6110*-based restriction fragment-length polymorphism (RFLP) pattern and contain 9 of 43 polymorphic spacer sequences in the chromosomal DR locus by spoligotyping (21).

We report the genotypic characterization and spread of predominantly MDR *M. tuberculosis* strains in a prison in Azerbaijan. Along with IS*6110*-based RFLP, spoligotyping, and drug-susceptibility profiles, we have determined the type of mutation in the *rpoB* gene responsible for resistance to rifampin (RMP).

Methods

Patients

At the beginning of the ICRC program, 65 male patients 19 to 55 years of age were included in the study. All were from Azerbaijan, and their terms of imprisonment ranged from 6 months to 20 years. Once diagnosed as having smear-positive pulmonary TB, they were transferred to the Central Penitentiary Hospital in Baku, the referral hospital for the Azerbaijan prison system. No clinical histories were available for the time before the ICRC intervention. Personal characteristics have largely been provided by the prisoners themselves (Table). Patients were classified at admission on the basis of their self-recorded anti-TB drug intake as nonresponders (patients who were treated with single or multiple drugs for >1 month and continued to be smear-positive

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Table. Characteristics of the cohort of prison inmates with tuberculosis, Azerbaijan

Strain no.	Age of patient (years)	Body mass index	Patient group[a]	Onset of symptoms	Previous treatment (before enrollment in ICRC program)[b]
7933	25	18.2	A	Aug 1996	R,E,S
7887	40	--	A	Jan 1995	H,R,E,S
7909	40	16.1	A	Jun 1995	S (4 mo. in 1995) S (1 mo. in 1996)
7920	22	16.3	A	Mar 1995	H(?),R(?),S
5695	22	17.2	B	Feb 1996	S
5698	43	--	A	1976	R,E
7101	20	--	B	Jan 1996	S
5691	26	--	A	Feb 1995	S
7915	21	16.2	B	Mar 1996	None
7922	33	16.1	A	Spring 1996	H,R,E,S
5689	24	19.7	A	1993	H,R,E,S (1 to 2 mo.)
7924	40	17.4	B	Apr 1996	H,R,E,S
7934	36	--[c]	A	Jul 1996	S
7908	32	16.1	B	Mar 1996	R,E,S
7930	21	16	B	Aug 1996	R,E,S
7935	23	17.3	B	Mar 1996	R
7925	22	19.3	A	Feb 1996	S (monotherapy initially); R,E,S (2 to 3 mo.)
7931	21	16	B	Jul 1996	R,S
7914	34	15.7	A	Nov 1995	R,E,S (not completed)
5713	25	15.6	B	Dec 1995	H,R,E,S
7910	32	17.5	A	Feb 1996	S,E (not completed)
7906	22	19.5	B	Aug 1996	R,S
5719	32	--	C	1991	H,R,E,S (9 mo. in 1991)
7923	34	13.8	B	Jan 1996	R,E,S
7899	33	15.8	A	Jan 1996	H (4 mo.), R (10 d), E (4 mo.), S,Z (4 mo.) (not completed)
5684	29	17.2	A	1993	H,R,E,S (in 1994) S (in 1995)
7900	?	20.2	A	Jul 1995	H,R,S (in 1995) S (in 1996)
5697	19	17.4	B	Mar 1996	none
5692	22	--	A	Feb 1995	H,R,E,S
7916	32	18.2	A	1994	H,R,E,S,K (several $\leq$3-mo. intervals of treatment)
7932	22	18.1	C	Nov 1993	H,R,E,S,K (several intervals of treatment: 1 mo., 4 mo., 12 mo.)
5700	34	18	B	Feb 1996	none
5702	26	18.1	A	Sep 1995	S
5706	23	--	B	Feb 1996	S
5716	24	19	B	Feb 1996	R,S
7888	30	--	A	Oct 1994	H,R,E,K (<2 mo. in 1994) H,R (in 1995)
5693	26	17.2	B	Jan 1996	S
5722	26	17.1	B	Dec 1995	H,R,E,S,K
7895	19	18.5	B	Jun 1996	H,R,S
5724	37	17.3	B	Dec 1995	H,R,E,S
7886	46[c]	--	A	Mar 1995	R,E,S (not completed)
7917	55	--	C	1987	R,E,S (11 mo.)

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Strain no.	Age of patient (years)	Body mass index	Patient group[a]	Onset of symptoms	Previous treatment (before enrollment in ICRC program)[b]
7933	25	18.2	A	Aug 1996	R,E,S
5718	20	17.6	B	Oct 1995	H,K
7889	?	--	A	?	?
7921	22	19.4	B	Jul 1996	S
7926	20	--[c]	B	Aug 1996	none
7918	33	16.4	B	Jul 1996	H,R,E,S
7927	32	18.4	A	Mar 1996	H,E,S (not completed)
7904	21	16.8	B	May 1996	H,S
7929	47	26.3	A	May 1996	R,E,S (not completed)
7894	28	17.3	B	Apr 1996	None
7897	20	13.8	A	Jan 1996	R,E,S (not completed)
7905	26	18.6	B	Apr 1996	S
7907	19	14	B	Dec 1995	H,S
7902	25	19.2	A	1995	H,S (6 mo.) R,E, (7 d)
5686	29	--	A	May 1995	H,R,E,S (not completed)
5694	29	17.3	A	Jan 1995	H,S (1 mo. in 1995) E,S (1 mo. in 1996)
5726	44	16.9	A	?	H,R,E,S (3 to 4 mo. in 1991) H,R,E,S (in 1995)
7911	34	17.9	B	Mar 1995	R,E,S,K
5688	37	--	A	Sep 1994	R,E,S
5687	41	20.3	B	Feb 1996	E,S
5729	40	16.3	C	1994	R,S(3 mo. in 1994) S (in 1996)
7890	19	--	A	1994	H,E,S (3 mo. in 1994) R,E,S (in 1996)
5699	22	--	B	Mar 1996	H,S

[a]Group A = nonresponders; Group B = new cases (never treated or treated for <1 month); Group C = relapsed cases
[b]H, isoniazid; R, rifampin; E, ethambutol; S, streptomycin; Z, pyrazinamide; K, kanamycin
[c]Patient too weak to be weighed

[group A; n = 30]), new cases (smear-positive patients who had never been treated or were treated for <1 month [group B; n = 31]), and relapsed cases (patients who had active TB and were treated and considered clinically cured but became smear-positive again [group C; n = 4]).

Microbiologic Diagnosis and Drug Susceptibility Testing

Sputum specimens were analyzed for the presence of acid-fast bacilli (Ziehl-Neelsen staining) by the local microbiology laboratory, which was supervised by the ICRC. Processing of specimens, culture, and identification of *M. tuberculosis* were done in Zurich or Antwerp according to standard procedures (22). Susceptibility testing of *M. tuberculosis* to primary drugs (isoniazid, RMP, ethambutol, and pyrazinamide) plus streptomycin was performed in liquid medium by the radiometric BACTEC 460 TB system (Becton Dickinson, Sparks, MD) (23) and confirmed by the agar proportion method (22).

Molecular Characteristics of RMP Resistance

All *M. tuberculosis* isolates were analyzed by the INNO-LiPA-Rif TB Assay (Innogenetics, Ghent, Belgium) (24). This reverse hybridization-based line probe assay carries one oligonucleotide probe for the detection of *M. tuberculosis* com-plex strains and nine probes to detect nucleotide changes in the relevant part of the *rpoB* gene.

Molecular Epidemiology

P*vu*II-digested DNA of *M. tuberculosis* was probed with the insertion element IS*6110* according to the standardized protocol of van Embden et al. (25), with some modifications (26). "Cluster strains" had 100% identical RFLP patterns.

Cluster Analysis

IS*6110*-based RFLP patterns were analyzed by GelCompar software (Windows 95, version 4.0; Applied Math, Kortrijk, Belgium) with an HP ScanJet 4c (Hewlett Packard, Greeley, CO; scanned at 190 dpi). The mobility of the IS*6110* fragments was compared with the molecular weight marker lambda-DNA *Eco*RI/*Hin*dIII (Roche Diagnostics, Rotkreuz, Switzerland), which was used as external size marker. In addition, the accuracy of the procedure was tested by comparing the IS*6110* banding pattern of *M. tuberculosis* isolate Mt14323, which was present on each autoradiograph. Patterns were compared by the UPGMA clustering method with the Dice coefficient (1.2%). Spoligotype patterns (27) generated from all 65 isolates were compared visually.

Results

Except for two patients in whom the first symptoms of TB had appeared 9 and 20 years previously, the patients had recent onset of TB disease (Table). Most prisoners were substantially undernourished (as indicated by low body mass indices) and in poor clinical condition, many with unilateral or bilateral pulmonary infiltrates and cavities. Most of the nonresponding patients (group A) had been treated inadequately before the ICRC intervention (Table).

Based on morphologic and biochemical characteristics, all 65 isolates were identified as classical *M. tuberculosis*. Hybridization of *Pvu*II-digested chromosomal DNA with the 245-bp fragment of IS*6110* resulted in variable patterns, although to a limited degree (Figure 1). Within a cluster (each cluster comprising a minimum of 2 and a maximum of 10 patients), isolates had identical IS*6110* patterns. Overall,

we found 11 clusters containing a total of 33 isolates. Apart from cluster 1, which consisted of 2 isolates with 10 IS*6110* copies, the isolates belonging to clusters 2 to 11 were characterized by 15 to 18 IS*6110* fragments and similar banding patterns (similarities of >90% for clusters 2 to 9 and ≥80% for clusters 10 and 11 with the former group). In addition to the 31 isolates of clusters 2 to 11, 15 other isolates of *M. tuberculosis* displayed similar IS*6110* patterns, differing in the presence or absence of one or two bands or in the IS*6110* fragment length (Figure 1). These 15 isolates were highly related (≥68%) to those of clusters 2 to 11. DNA fingerprints of the 31 isolates in clusters 2 to 11 and the 15 closely associated isolates strongly resembled the Beijing genotype of *M. tuberculosis* (Figure 1, asterisk).

Spoligotyping grouped those 46 isolates into one genotype with identical spoligotypes that lacked all but 9 of the 43 DR spacers and thus resembled the Beijing family of *M. tuberculosis.* The remaining 19 isolates of the patient cohort resulted in different, mostly individual spoligotypes (Figure 2). While cluster 1 was confirmed by spoligotyping, other isolates, mostly with a high degree of similarity in their RFLP patterns, were also grouped together (isolates 7887/5695/7920; isolates 5691/5689; isolates 7922/7915; and isolates 7908/7935; Figure 2).

Regardless of Beijing or other types, the susceptibility patterns differed, ranging from fully susceptible to resistant to one or more drug(s). Overall, 52.3% of the strains isolated from the cohort (Figure 2) and 60.9% (28/46 isolates) in the group of Beijing type strains were MDR. While none of the RMP-susceptible strains carried a mutation in the relevant part of the *rpoB* gene, all strains resistant to RMP (by growth-based susceptibility testing) were correctly identified by the INNO-LiPA-Rif assay. For 22 of them, the type mutation was detected: D516V (R2; n = 1), H526Y (R4a; n = 2), H526D (R4b; n = 1), and S531L (R5; n = 18). S531L was predominant in the Beijing family (17/28 RMP^R isolates = 60.7%; Figure 2. MDR strains of *M. tuberculosis* were found in 19 of 30 patients in group A, 14 of 31 patients in group B, and 1 of 4 patients in group C. Full drug susceptibility was also seen in all groups of patients (3 of 30 in group A, 7 of 31 in group B, and 1 of 4 in group C).

Conclusion

Analyzing the TB patients in the Central Penitentiary Hospital in Baku was complicated by constraints and biases inherent in the prison environment. Clinical information on the prisoners was limited and mainly based on self-reported data. Conclusions based on analysis of 65 of the approximately 300 TB patients in that hospital are largely fragmentary and may not be truly representative. However, enrolling more patients into our pilot study was not considered, mainly because of frequent transfer of prisoners and high mortality rates. When the DOTS program was implemented by the ICRC, many of the TB patients were either untreated or had received inadequate drug regimens for years.

Although we analyzed a limited number of patients, our study demonstrates that most strains (70.8%) belonged to the Beijing family of *M. tuberculosis* and that multidrug resistance was a serious problem (52.3%) in this setting. The Beijing genotype, characterized by 15 to 18 IS*6110* copies and a specific spoligotype pattern, was identified in 10 of the 11 clusters (2 to 11) and included isolates of 31 patients, plus
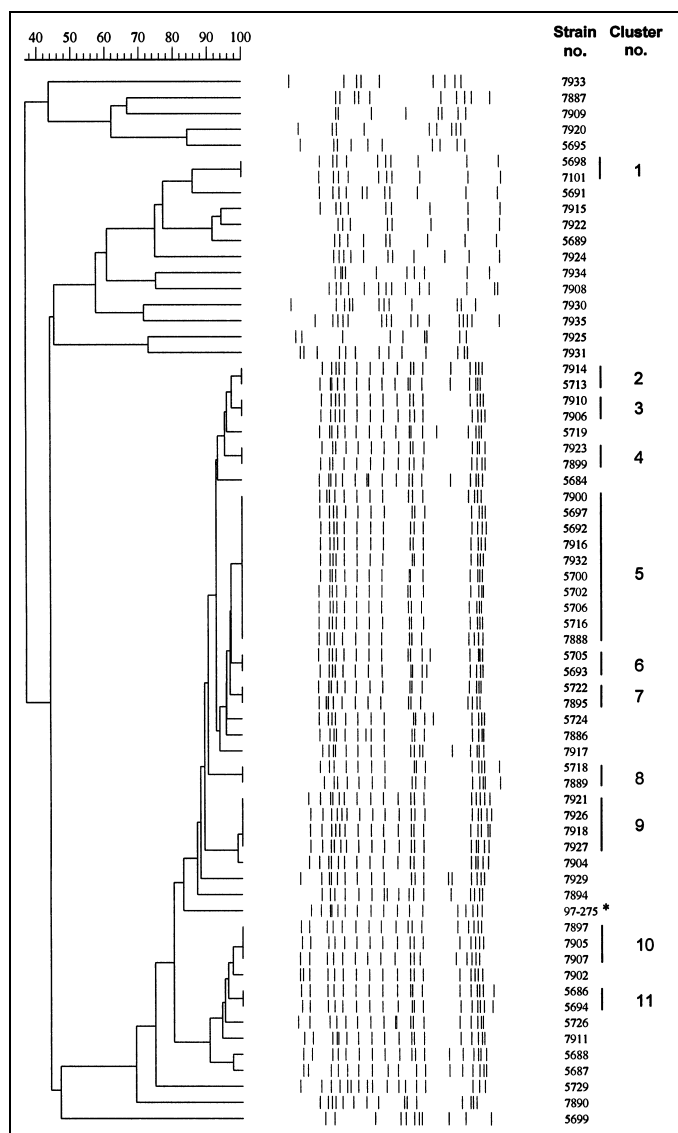
Figure 1. Computer-generated lane maps of genomic fingerprints of *Mycobacterium tuberculosis* strains isolated from the patient cohort (n = 65). *Pvu*II-digested chromosomal DNA was probed with IS*6110*. Clusters 1 to 11 are indicated by vertical lines at the right margin. Asterisk indicates strain 97-275 (Beijing clone, obtained from K. Kremer, RIVM/NL) for comparison. Strains 7914 through 5687 show the Beijing genotype restriction fragment-length polymorphism pattern. The molecular weight marker and internal reference strain of *M. tuberculosis* (Mt 14323) are not shown.

Scale: 40 50 60 70 80 90 100

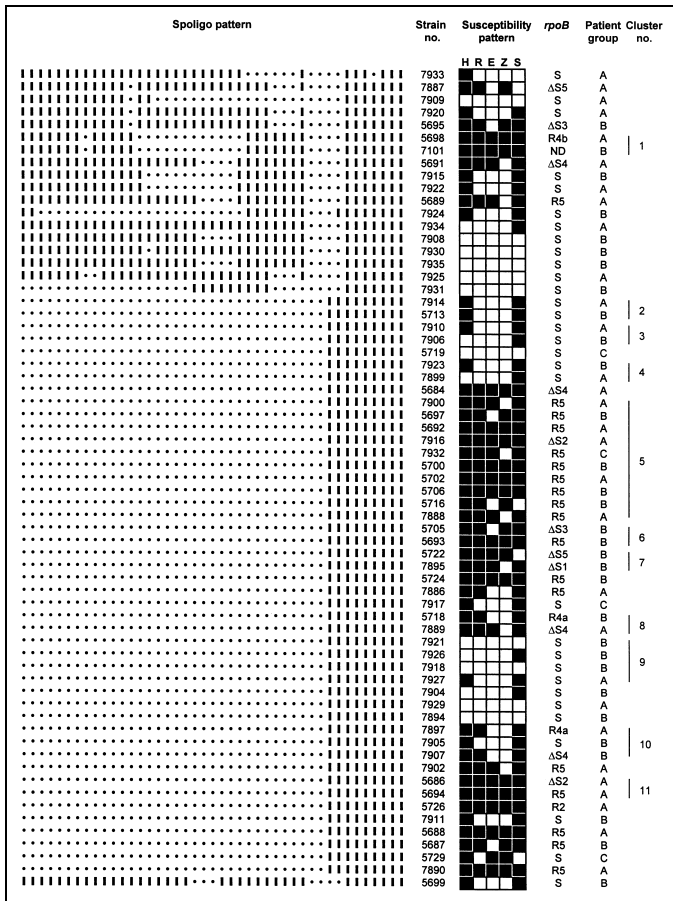| Strain no.	Cluster no.
7933	
7887	
7909	
7920	
5695	
5698	
7101	1
5691	
7915	
7922	
5689	
7924	
7934	
7908	
7930	
7935	
7925	
7931	
7914	2
5713	
7910	3
7906	
5719	
7923	4
7899	
5684	
7900	
5697	
5692	
7916	
7932	
5700	5
5702	
5706	
5716	
7888	
5705	6
5693	
5722	7
7895	
5724	
7886	
7917	
5718	8
7889	
7921	
7926	9
7918	
7927	
7904	
7929	
7894	
97-275	*
7897	
7905	10
7907	
7902	
5686	11
5694	
5726	
7911	
5688	
5687	
5729	
7890	
5699	

Figure 2. Spoligotypes of the 65 strains of *M. tuberculosis* analyzed, drug susceptibility patterns, and type of mutation on the *rpoB* gene conferring rifampin resistance (*rpoB*). H = isoniazid; R = rifampin; E = ethambutol; Z = pyrazinamide; S = streptomycin. Black fields indicate resistance; blank ones susceptibility. S = wild type (RMP^S); mutations (RMP^R): ΔS1; DS2; ΔS3; ΔS4; ΔS5; R2; R4a; R4b; R5; ND = not done. Group A = nonresponders; Group B = new cases; and Group C = relapsed cases.

(30,31). Recently, Naroditskaya et al. (unpub. data) studied 40 MDR-TB isolates from inmates of a prison in Tomsk (Russia) and reported that as many as 77.5% of the isolates had a substitution of ser^{531} ≥leu. Of these isolates, 15 had an RFLP pattern (W148) specific for the so-called W-strain family, which is highly drug-resistant and belongs to the phylogenetic lineage of the Beijing family. W and W-like strains have been associated with large MDR-TB outbreaks in the United States (16,17) and elsewhere (e.g., South Africa [19]). With high disease and mortality rates, MDR outbreaks caused by the W strain family and its variants have mainly affected HIV-infected persons (17,18) but affect HIV-negative persons as well (19,32). The HIV status of our cohort remained unknown, since the prisoners had not been tested. We assume that HIV infection has had little influence, since its incidence in the general Azeri population is low (33).

The difficulty in establishing transmission links among prisoners is well known. Although ample clinical information about their patients was available, Jones et al. (8) were unable to identify specific person-to-person lines of transmission among most inmates in a North American jail. Having been unable to establish accurate links among inmates or to define index patient(s) in the Baku Penitentiary Hospital, we can only hypothesize possible modes of transmission; for example, some patients may have contracted TB in the community long before they were imprisoned. Political constraints prevented us from studying TB strains in the Azeri population, which would have allowed us to analyze the molecular genetic composition of the strains circulating in the community, as well as to estimate the prevalence of the Beijing genotype strains. Acquisition of TB disease within the prison walls is, however, more likely. Identical RFLP patterns and spoligotypes, as well as identical susceptibility patterns, including the same point mutation in the *rpoB* gene (e.g., isolates 5692, 5700, 5702, 5706, 7932, and 7888 of cluster 5) suggest recent person-to-person transmission of TB. Nevertheless, we could not establish that the isolates of the large cluster carrying an S531L mutation are all derived from the same index strain. Since this mutation is by far the most frequent one, it could also have arisen independently in some of these strains.

Recent transmission within the prison is questionable for those cluster strains that are identical by RFLP, spoligotype and susceptibility profiles but have distinct mutations in the *rpoB* gene (e.g., isolate 7916 [cluster 5: ΔS2] versus all other isolates of this cluster [R5]; isolates 7897 and 7907 [cluster 10: R4a and ΔS4]; isolates 5686 and 5694 [cluster 11: ΔS2 and R5]). Thus, in all these cases TB is most likely not the result of recent transmission from a single source, which would require an RMP-susceptible progenitor strain. The fact that different susceptibility patterns were detected among the Beijing strains may indicate that transmission in the prison primarily involved RMP-susceptible strains, with resistance to RMP (and other drugs) evolving gradually in individual patients.

From this study, several conclusions can be drawn: MDR-TB is a substantial problem in Azeri prisons; the Beijing clone of *M. tuberculosis* was found in all three patient groups (nonresponders, new cases, and relapsed cases) and had a high rate of multidrug resistance; modes of transmission remain unclear, i.e., TB may have been acquired in different ways (outside or inside the prison); and MDR-TB in

isolates from an additional 15 patients that had a high similarity with the cluster strains on IS*6110*-based DNA fingerprinting. Sharing most of the IS*6110*-containing restriction fragments and the polymorphism associated with other repetitive DNA elements (28), the Beijing clone *of M. tuberculosis* has been isolated predominantly from patients in the People's Republic of China and neighboring Asian countries such as Mongolia, Korea, and Thailand (20). The emergence of the Beijing clone is now also well documented in Vietnam: Anh et al. have shown that 304 (54%) of 563 isolates studied were of that genotype and associated with younger patients and, hence, with active transmission (29).

In the three patient groups, only 11 isolates of *M. tuberculosis* were fully drug susceptible (2 patients in group A; 7 patients in B; and 1 patient in C). Dual resistance to isoniazid and streptomycin was seen in 10 cases (4 in A and 6 in B). Multidrug resistance was particularly associated with the Beijing genotype (28/46 = 61% versus 6/19 = 31% with the non-Beijing type) and, as expected, most frequent in patients of group A (19 in A, 14 in B, and 1 in C). For strains of the Beijing group whose mutation types could be determined, substitution at ser^{531} (mutation type R5) predominated. Published reports indicate that this mutation is the most common point mutation conferring RMP resistance

this cohort may result from recent transmission in the prison, but may also have evolved as a result of longstanding, inadequate treatment regimens.

Unless WHO/IUATLD-recommended treatment schemes are followed, the problem of TB, in particular of MDR-TB, may become more widespread (8). In response, both WHO and ICRC have developed guidelines for managing TB in prisons (2). Special emphasis has to be put on settings where TB strains have a high degree of resistance, since regimens with first-line antibiotics may not be sufficient, as demonstrated by Coninx et al. (34). To achieve TB control in prisons, multiple goals need to be attained: efficient diagnosis to permit early case finding, prompt initiation of standardized treatment, monitoring of patient response to treatment, and provisions for ample supply of anti-TB drugs that meet quality standards.

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